J Chem Ecol (2013) 39:1070–1080
DOI 10.1007/s10886-013-0303-2

Comparisons of Contact Chemoreception and Food Acceptance by Larvae of Polyphagous *Helicoverpa armigera* and Oligophagous *Bombyx mori*

Hui-Jie Zhang · Cécile P. Faucher · Alisha Anderson · Amalia Z. Berna · Stephen Trowell · Quan-Mei Chen · Qing-You Xia · Sylwester Chyb

Received: 13 January 2013 / Revised: 3 May 2013 / Accepted: 7 May 2013 / Published online: 19 June 2013

Abstract We compared food choice and the initial response to deterrent treated diet between fifth instars of *Helicoverpa armigera*, a polyphagous generalist pest, and *Bombyx mori*, an oligophagous specialist beneficial. *Bombyx mori* was more behaviorally sensitive to salicin than to caffeine. The relative sensitivities were reversed for *H. armigera,* which was tolerant to the highest levels of salicin found in natural sources but sensitive to caffeine. A single gustatory receptor neuron (GRN) in the medial styloconic sensillum of *B. mori* was highly sensitive to salicin and caffeine. The styloconic sensilla of *H. armigera* did not respond consistently to either of the bitter compounds. Phagostimulants also were tested. *Myo*-inositol and sucrose were detected specifically by two GRNs located in *B. mori* lateral styloconic sensillum, whereas, in *H. armigera*, sucrose was sensed by a GRN in the lateral sensillum, and *myo*-inositol by a GRN in the medial sensillum. *Myo*-inositol responsiveness in both species occurred at or below 10^{-3} mM, which is far below the naturally occurring concentration of 1 mM in plants. Larval responses to specific plant secondary compounds appear to have complex determinants that may include host range, metabolic capacity, and gustatory repertoire.

Keywords Food preference · Gustation · Styloconic sensillum · Gustatory receptor neuron · Sugar response · Bitter response

Electronic supplementary material The online version of this article (doi:10.1007/s10886-013-0303-2) contains supplementary material, which is available to authorized users.

H.-J. Zhang · Q.-M. Chen · Q.-Y. Xia (✉)
State Key Laboratory of Silkworm Genome Biology, Southwest University, Chongqing 400715, China
e-mail: xiaqy@swu.edu.cn

H.-J. Zhang
e-mail: huijie.zhang724@gmail.com

H.-J. Zhang · C. P. Faucher · A. Anderson · A. Z. Berna · S. Trowell · S. Chyb (✉)
CSIRO Food Futures Flagship and CSIRO Ecosystem Sciences, Canberra, ACT 2601, Australia
e-mail: sylwester.chyb@ymail.com

Present Address:
C. P. Faucher
Department of Neuroscience, University of Arizona, Tucson, AZ, USA

Introduction

Phytophagous insects may be categorised as polyphagous, oligophagous, or monophagous, depending on the range of host plants they will accept. In Lepidoptera, a sophisticated olfactory system helps females navigate towards a host by using volatile chemical cues. The larval gustatory system is adapted to mediate acceptance or rejection of plant tissues following drumming and test bites (Chapman 1982; Devitt and Smith 1985). The mechanism of food choice and preference in caterpillars is of great interest, since many economically important agricultural pests are members of the order Lepidoptera, and most lepidopteran larvae have specialised feeding habits. For example, larvae of the domesticated silkworm, *Bombyx mori*, feed mainly on mulberry leaves and, only very rarely, on a few other species of Moraceae, Compositae, or Ulmaceae (Legay 1958; Tanaka 1943). In contrast, *Helicoverpa armigera*, a widely distributed phytophagous lepidopteran, is a typical polyphagous insect, which feeds on at least 200 species from 30 plant families including members of the Solanaceae, Gramineae and Leguminosae (Chin et al. 1962; Xu et al. 1958).

Caterpillars with a restricted host plant range are behaviorally more sensitive to deterrents, bitter plant secondary

metabolites, than polyphagous caterpillars (Bernays and Chapman 1987, 1994; Bernays et al. 2000; Jermy 1964), although this is not necessarily true in other orders (Eichenseer and Mullin 1997). Most behavioral comparisons are based on phylogenetically closely related species with different host ranges (Bernays and Chapman 1987, 2000; Bernays et al. 2000). A model has been developed that seeks to explain these differences in terms of central nervous system processing rather than differences in sensory input (Schoonhoven and Blom 1988), and there is experimental support for such a model (Bernays and Chapman 2000).

In caterpillars, food evaluation is performed by gustatory organs located on the mouthparts. These are paired sensilla styloconica on the maxillary galea, basiconic sensilla on the top of the maxillary palps, as well as sensilla on the epipharynx (Dethier 1937; Ishikawa and Hirao 1961; Ma 1972; Schoonhoven 1969; Waldbauer and Fraenkel 1961). The two sensilla styloconica seem to play a particularly important role in acceptance of host plants (Schoonhoven 1987). Each styloconic sensillum houses four gustatory receptor neurons (GRNs) with specific response spectra for plant compounds (summarized by Schoonhoven and Van Loon 2002; Shields 2009). Typically, some of the neurons respond to phagostimulants, primary plant metabolites like sugars and amino acids that evoke feeding. Other GRNs are activated by deterrents, secondary plant metabolites commonly bitter to humans that mediate food aversion. Secondary compounds are highly diverse and often characteristic of one or a few plant families. Feeding is not determined by the simple presence or absence of specific compounds, but rather by a balance between deterrent and stimulatory compounds (Chapman 2003).

In the maxillary gustatory system of the silkworm, one GRN in the lateral styloconic sensillum is sensitive to sucrose, and the other three respond to *myo*-inositol, glucose, or salts, respectively (Ishikawa 1963, 1966; Ishikawa and Hirao 1963). However, one GRN in the medial sensillum is sensitive to bitter compounds, and the others respond to water, salts, acids, ecdysone, or 20-hydroxyecdysone (Descoins and Marion-Poll 1999; Tanaka et al. 1994). A few gustatory sensilla also are found in the maxillary palps, which are involved in food detection and selection (Ishikawa et al. 1969). Synergistic as well as suppressive interactions have been observed at the peripheral level, central nervous system, and behavioral levels (Hsiao and Fraenkel 1968; Ishikawa 1966; Ishikawa et al. 1969; Thorsterinson 1960). Compared with *B. mori*, *H. armigera* has two GRNs in each styloconic sensillum that respond to sucrose and alanine separately. The other two GRNs detect deterrents from plant saps (Simmonds and Blaney 1991). A more recent study has reported that one neuron in the medial styloconic sensillum responds to *myo*-inositol rather than sucrose (Tang et al. 2000).

Here, by using identical stimuli presented under the same conditions, we compared larval feeding behavior and the sensory physiology of the sensilla styloconica of fifth instar *H. armigera* with those of *B. mori*. As is usual in this field, we restricted our study to fifth instars for experimental convenience. We found that *B. mori* is approximately two orders of magnitude more behaviorally sensitive than *H. armigera* to salicin. Surprisingly, however, *B. mori* was much less sensitive than *H. armigera* to caffeine. In both cases, the more sensitive species responded in the naturally occurring range for the bitter compounds tested. The styloconic sensilla of *B. mori* were sensitive to detect both bitter compounds below the behavioral threshold, but detection of these deterrents was not observed in *H. armigera*. Regarding sugars, we found that larvae of both species could detect the plant sucrose and *myo*-inositol at or close to physiologically relevant concentrations. Our findings may provide more data for a more species-specific understanding of the physiological and molecular characteristics of food determination in caterpillars.

Methods and Materials

Insects *Bombyx mori* larvae used in the 24 h feeding inhibition test were reared on mulberry leaves at 25 °C in the State Key Laboratory of Silkworm Genome Biology, Southwest University, Chongqing, China (Xia et al. 2004). Larvae used in behavioral studies on initial contact with food and for electrophysiological recording were maintained on mulberry leaves at 25 °C in CSIRO Ecosystem Sciences, Canberra, Australia. *Helicoverpa armigera* larvae were reared on artificial diet, according to Teakle and Jensen (1985), as modified by Mahon et al. (2007), and maintained in CSIRO Ecosystem Sciences. The strain is a combination of lab strains dating back to 1988 that has been regularly outcrossed to "field" material from Australia.

Scanning Electron Microscopy (SEM) Heads were excised from newly molted fifth instars of *B. mori* and *H. armigera* and fixed in 2.5 % glutaraldehyde for 3 h. They were dehydrated through a graded alcohol series and air dried at 25 °C, before being mounted on a specimen holder with double-sided adhesive tape and sputter-coated with gold. The preparations were examined with a Hitachi H-5700 (Tokyo, Japan) SEM at 15 kV.

Chemicals Salicin and caffeine were chosen as antifeedants. In nature, caffeine concentration varies in the range of 6.8–21 g/kg (35–108 mM) in fresh tea leaves to 8–18 g/kg (41–92 mM) in fresh coffee beans, both naturally rich sources of the compound (Nathanson 1984). Salicin occurs at up to 0.5 g/kg (1.7 mM) in fresh leaves of aspen and similar levels are found in willow (Lindroth et al. 1988; Smiley et al. 1985). These values likely would represent the higher end

of the natural range in plants. Sucrose and *myo*-inositol were chosen as sweet compounds. These chemicals and KCl were purchased from Sigma at ≥99 % purity. All tested compounds were dissolved in 50 mM KCl for electrophysiology, which served as electrolyte in both electrodes, and in ultrapure water for the behavioral tests. The antifeedant and sweet chemicals were selected based on their common use in experiments with lepidopteran insects (Chapman 2003; Schoonhoven and Van Loon 2002). The ranges of concentrations chosen for physiological and behavioral experiments were those found to span the dynamic range of responses, using initial ranging experiments. The concentrations of sucrose and *myo*-inositol tested electrophysiologically were 10^{-5}, 10^{-3}, 0.1, 1, 50, and 100 mM. Salicin and caffeine were tested at 10^{-5}, 10^{-3}, 0.1, 1, 5, and 10 mM. For behavioral assays, concentrations of salicin were 0.1, 1, 5, 10, 50, 100, and 500 mM and of caffeine were 0.01, 0.1, 1, 5, 10, 100, and 500 mM.

Behavioral Assay Newly molted fifth instars were starved for 24 h before being tested on artificial diet. Ten *B. mori* larvae were placed in a dish containing a 2 g disc (~1.5 cm diam.) of artificial diet containing various concentrations of caffeine and salicin (mulberry flour 20 g, sucrose 4.75 g, *myo*-inositol 1.62 g, agar 1.5 g, X grams of caffeine/salicin, water 100 ml) Ten *H. armigera* were tested individually to prevent cannibalism as well as on their own diet to avoid maladjustment. The *H. armigera* artificial diet (Mahon et al. 2007), containing caffeine or salicin, was presented to larvae as for *B. mori* in 2 cm discs. Controls were offered untreated food discs. After 24 h, discs were removed and weighed. Assays were repeated three times. The 'feeding inhibition index' was calculated as follows:

$$\text{Feeding inhibition index} = 100 \times \left[\left(\text{Mass consumed(control disc)} - \text{Mass consumed(treated disc)}\right) \Big/ \text{Mass consumed(control disc)}\right]$$

An index close to 100 % indicates a strong deterrent effect, whereas close to 0 % indicates a weak effect.

We also observed the behavior of newly molted fifth instars on their initial encounter with diet containing aversive stimuli (same as above). Fifteen larvae for each treatment starved for 24 h were tested individually. We recorded whether the larvae either tasted the food, which included drumming and biting but not eating, or ate the food. Eating was defined as continuous feeding for at least 10 sec. We also recorded how long larvae spent eating within the first 20 min after encountering food.

Electrophysiological Recording Tip-recordings were performed on the medial and lateral styloconic sensilla of fifth instar *B. mori* and *H. armigera* (aged 2 d after molting), as described by Ishikawa (1963) and van Loon (1990) with some modifications. Larval heads were cut from the thorax, and the reference glass electrode, filled with 50 mM KCl, was inserted into the head until pressure caused the mouthparts to open. A recording glass electrode filled with tastant in 50 mM KCl solution was brought into contact with the tip of a sensillum under a dissecting microscope. Electrophysiological activity was recorded with a Tasteprobe amplifier (Marion-Poll and Van der Pers 1996) linked to a data acquisition controller (IDAC-4: Syntech, The Netherlands) and a computer equipped with the AutoSpike-32 software (Syntech). Responses were recorded from both the medial and lateral sensilla on the same side of the head. Stimuli lasted 2 sec and were separated by an interval of 2 min to allow for recovery and to minimize adaptation. Sensilla were washed between recordings by bringing their tip into contact with a solution of 50 mM KCl, and were repeated twice. The order of stimulation was random except for 50 mM KCl, which was always tested first.

Only recordings from the same side of each insect were used for analysis. Analysis of the spikes was performed by using AutoSpike-32 (Syntech) based on the amplitude. The response intensity (spikes/sec) was determined by automated counting of the number of spikes in the first second. The responses to tested substances were generally quite stable over time, except for sucrose and *myo*-inositol where the responses decreased noticeably after 1 sec in *H. armigera*. In addition, as automated counting and classification is not always error free, we revised results by visually counting and distinguishing spikes on the basis of their amplitudes, firing regularity, and shapes (reflecting doublets) (Hiroi et al. 2004). The presence of doublets was used to determine simultaneously occurring spikes, which may appear as a larger spike than usual. Data are presented as means ± standard error of the means from the sensilla of at least five different larvae.

Principal Components Analysis Principal Component Analysis (PCA) was employed to compare the electrophysiological responses of the GRNs in lateral and medial styloconic sensilla to different concentrations of various tastants in *B. mori* and *H. armigera*. The correlation matrix of electrophysiological responses of the lateral and medial styloconic sensilla to all concentrations of the tastants (sucrose, *myo*-inositol, salicin and caffeine) were submitted for PCA analysis

by using Unscrambler software (version 9.1, CAMO PROCESS AS, Nedre Vollgate, Norway). Correlation matrix was considered so that the responses with the highest variance would not dominate the first principal component. The scores were graphed, and vectors were used to indicate the tastants that characterise differences between the sensilla. (PCA seeks to reduce the number of variables that need to be considered to a smaller number of indices, called principal components. The first principal component accounts for the largest quantum of variance among samples. Subsequent principal components account for successive amounts of the total variance in the data set and are uncorrelated with prior principal components).

Results

Behavioral Sensitivity to Plant Secondary Metabolites Both bitter substances inhibited feeding in both species, with some notable differences in sensitivities (Fig. 1). (Note that we were only able to obtain a robust dose–response fit for *B. mori* feeding on salicin. Estimates of the other antifeedant EC_{50} values are based on visual inspection of the curve fits). *Bombyx mori* was highly sensitive to the phenolic glycoside salicin with an EC_{50} of 4.9±2.5 mM. *Helicoverpa armigera* was 40 times more tolerant of salicin than *B. mori*, requiring concentrations of approximately 200 mM to inhibit 50 % of the feeding. *Bombyx mori* was moderately behaviorally sensitive (estimated EC_{50}≈100 mM) to the alkaloid caffeine. On the other hand, *H. armigera* was approximately ten-fold more sensitive to caffeine than *B. mori*, with an estimated EC_{50} of approximately 10 mM.

To exclude the possibility that feeding inhibition by the antifeedants is mediated by post-ingestion toxicity, we characterized the first encounter of larvae with deterrent treated diet (Fig. 2). Approximately 80 % of *B. mori* larvae ate on initial encounter with diet containing 1 mM salicin and control diet (Fig. 2a). Approximately half the larvae ate diet supplemented with 5 mM salicin, and none ate at 100 mM. Five and 10 mM caffeine resulted in approximately half the larvae eating the food, but the highest concentration tested strongly inhibited eating (Fig. 2b). Over the 20 min of the test, *B. mori* larvae spent less time eating on diet containing any level of caffeine or high concentrations of salicin than on control diet (Online Resource 1). A video of *B. mori* tasting and eating behavior is included in Online Resource 2. In contrast, for *H. armigera*, the presence of salicin did not alter the initial eating behavior (Fig. 2c), although larvae spent less time eating over the 20 min period compared to controls (Online Resource 1). With caffeine, *H. armigera* had very different initial responses (Fig. 2d). At 5 mM caffeine, approximately half the larvae ate immediately, and the other half only tasted the diet. At 10 mM and 500 mM concentrations of caffeine, *H. armigera* larvae bit and immediately spat out the food. Less time was spent eating over the 20 min period for all concentrations of caffeine, and effectively nothing was eaten at the higher concentrations (Online Resource 1). A video of *H. armigera* tasting and eating behavior

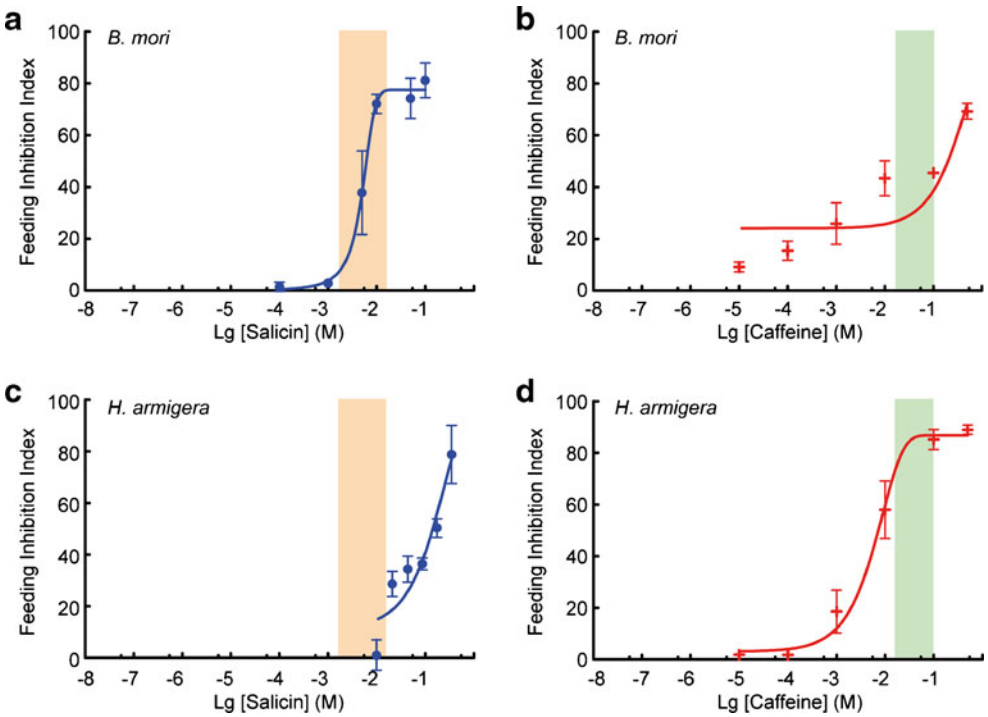

Fig. 1 Behavioral responses in *Bombyx mori* and *Helicoverpa armigera* larvae. *Bombyx mori* behavioral responses to salicin (**a**) and to caffeine (**b**). *Helicoverpa armigera* behavioral response to salicin (**c**) and to caffeine (**d**). Curves indicate the mean feeding inhibition (N=30). Curves were fitted with a variable slope nonlinear fit. Bars represent standard errors of the mean. The orange and green (*shaded*) areas on the graphs represent the range of concentrations encountered in rich natural plant sources of salicin and caffeine, respectively

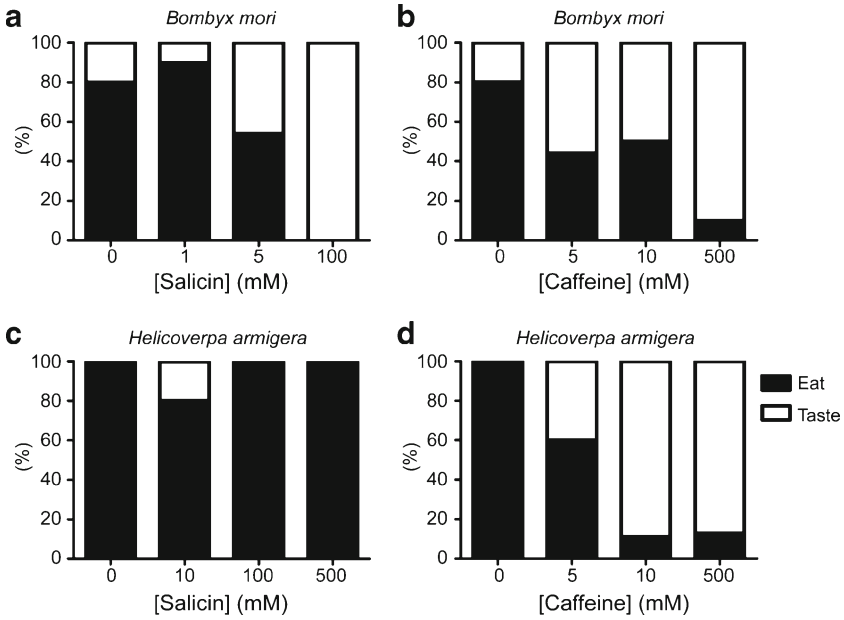

Fig. 2 Initial behavioral responses of *Bombyx mori* and *Helicoverpa armigera* larvae to diet supplemented with antifeedant. **a**, **b** Percentages of *B. mori* larvae that only tasted the diet (*no shading*) or ate (*black shading*) on first encounter with food treated with salicin (**a**) or caffeine (**b**) (N=10–15). **c**, **d** Percentages of *H. armigera* larvae that only tasted the diet (*no shading*) or ate (*black shading*) on first encounter with food treated with salicin (**c**) or caffeine (**d**) (N=10–15)

is included in Online Resource 3. These observations were, thus, highly consistent with the feeding inhibition data, and eliminate the need to invoke post-ingestion toxicity to explain the feeding inhibition observed in the first experiment.

Morphology of the Gustatory Sensilla of B. mori and H. armigera Scanning electron microscopy (Fig. 3) showed that the morphology of *H. armigera* maxillae is similar to that of *B. mori* and to the general plan seen in other lepidopteran larvae (Bernays and Chapman 1994; Grimes and Neunzig 1986; Ishikawa 1963). We observed no obvious differences in the locations of either the styloconic sensilla on the maxillary galea or of the basiconic sensilla on the maxillary palp.

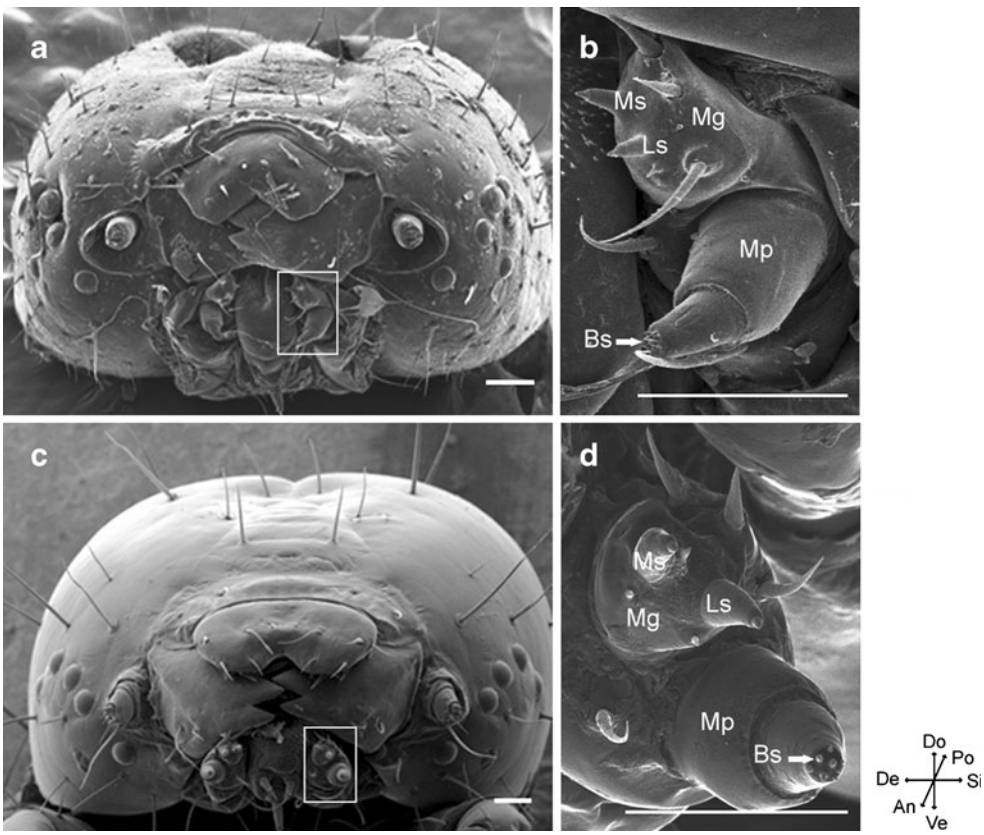

Fig. 3 Scanning electron micrographs of fifth instar heads showing maxillae. **a**, **c** Anterior images of *Bombyx mori* (**a**) and *Helicoverpa armigera* (**c**) heads. **b**, **d** Enlarged views of a maxilla of *B. mori* (**b**) and *H. armigera* (**d**). White rectangles indicate the location of maxillae. Mg, maxillary galea; Ms, medial styloconic sensillum; Ls, lateral styloconic sensillum; Mp, maxillary palp; Bs, basiconic sensillum. Do, dorsal; Po, posterior; Si, sinistral; Ve, ventral; An, anterior; De, dextral. Scale bar: 100 μm

Overall Analyses of the Neuronal Responses to Sweet and Bitter Compounds The PCA score plots (Fig. 4) show that the GRNs in the lateral and medial sensilla respond differently to the test compounds in the two species. Compositional loadings plotted as vectors show that GRNs in the lateral styloconic sensillum of *B. mori* are predominantly excited by sucrose and *myo*-inositol, whereas those in medial styloconic sensillum are excited by salicin and caffeine (Fig. 4a). In contrast, the plots for *H. armigera* show that GRNs in lateral sensillum respond to sucrose, while those in the medial sensillum respond primarily to salicin and *myo*-inositol (Fig. 4b).

Concentration Dependence of Bitter and Sweet Detection in Styloconic Sensilla In both species, the GRNs in each sensillum responded to at least one compound (Fig. 5). In most cases, the response appeared phasic-tonic, with the highest firing rate for the first 50–100 ms, followed by sustained

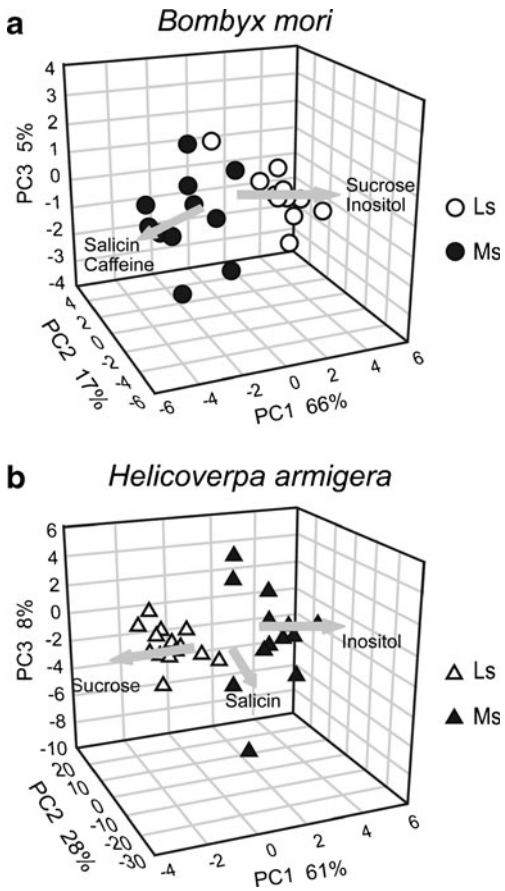

Fig. 4 Comparison of gustatory receptor neuron (GRN) responses from the lateral and medial styloconic sensilla of *Bombyx mori* and *Helicoverpa armigera* larvae. **a** PCA score plot of the GRNs responses to various concentrations of tastants in *B. mori* medial and lateral styloconic sensilla (N=6–13). **b** PCA score plot of the GRNs responses to various concentrations of tastants in *H. armigera* medial and lateral styloconic sensilla (N=5–13). *Grey arrows* indicate the compositional loadings for bitter and sweet compounds plotted as vectors. Ls, lateral styloconic sensillum Ms; medial styloconic sensillum

firing until the end of the two second stimulation (Online Resource 4). In the GRNs of *H. armigera* lateral sensilla, the spike amplitudes were larger during the first second of stimulation with sucrose, and then dropped noticeably (Online Resource 4). Similar results were observed in medial sensilla GRNs stimulated by *myo*-inositol. This phenomenon was not observed in *B. mori*.

The GRNs in the lateral styloconic sensilla of *B. mori* exhibited concentration-dependent increases in firing rate with sucrose and *myo*-inositol (Fig. 5a, b). However, the firing rate with 10 mM salicin or caffeine was not distinguishable from that with 50 mM KCl (Fig. 5a). In *H. armigera*, the spiking rate of GRNs increased with sucrose in the lateral sensillum and with *myo*-inositol in the medial sensillum (Fig. 5d, e, f). The GRNs in the medial sensilla of *B. mori* were sensitive to both salicin and caffeine and exhibited concentration-dependent responses to salicin and caffeine (Fisg. 5a, c). In contrast, the neuronal responses to bitter compounds in most *H. armigera* styloconic sensilla were low and not distinguishable from the control. Therefore, clear concentration-dependence curves could not be generated for *H. armigera*'s responses to salicin or caffeine.

Response of Individual GRN in B. mori and H. armigera Tip-recording aggregates the responses from the four chemosensory neurons in each of the styloconic sensilla. Judicious examination of spike traces allows some conclusions to be drawn about the neuronal basis of responses, although it is difficult to positively identify neuron types across recordings due to the variation among preparations.

In *B. mori* lateral styloconic sensilla, sucrose or *myo*-inositol each generated only one spike shape (Figs. 5a and 6a). When the two sugars were presented in a mixture, both spike shapes were observed with superposition of spikes visible, indicating that different neurons are sensitive to sucrose and *myo*-inositol (Fig. 6a, Online Resource 5). The amplitudes of spikes from the *myo*-inositol-sensitive neuron appear slightly higher than those from the sucrose-sensitive neuron. The responses of GRNs in *B. mori* lateral sensillum to salicin and caffeine showed spikes similar to those observed with 50 mM KCl (Fig. 5a, Online Resource 4).

In *B. mori* medial styloconic sensilla, the spikes generated by sucrose and *myo*-inositol were similar to those generated by KCl (Fig. 5a). Salicin and caffeine appeared to generate the same type of spikes, unlike the multiple spikes generated by the sugars or KCl (Fig. 5a). Indeed, the binary mixture of bitter compounds elicited only one type of spike, at higher frequency than with the individual components, but with no evidence of superposition, suggesting that in *B. mori* a single GRN responds to both salicin and caffeine (Fig. 6c, d).

In *H. armigera*, sucrose generated a distinct uniform spike type in a GRN of the lateral sensillum, and *myo*-inositol generated a distinct spike type in a GRN of the medial

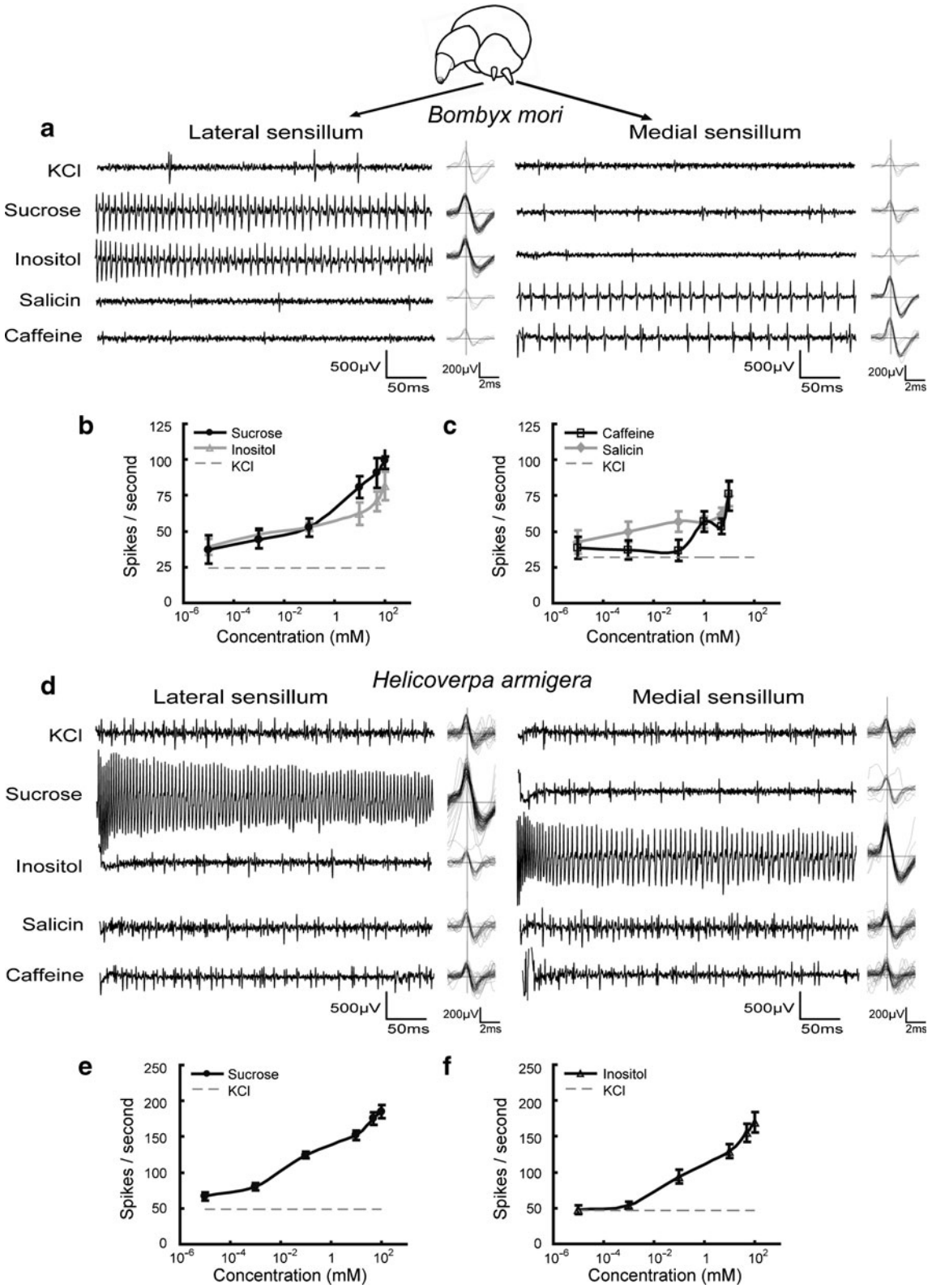

Fig. 5 Electrophysiological responses of *Bombyx mori* and *Helicoverpa armigera* larval gustatory receptor neurons (GRNs) to bitter and sweet stimuli. **a** Examples of spike traces from *B. mori* styloconic sensilla over the first 500 ms of stimulation, with spike shape superimposition. **b**, **c** Concentration-dependence of GRN responses to sucrose and *myo*-inositol in the lateral styloconic sensillum (N=13) and to salicin and caffeine in the medial styloconic sensillum (N=10). **d** Examples of spike traces from *H. armigera* styloconic sensilla over the first 500 ms of stimulation, with spike shape superimposition. **e**, **f** Concentration-dependence of GRN responses to sucrose in the lateral styloconic sensilla of *H. armigera* to sucrose (N=11) and to *myo*-inositol in the medial styloconic sensilla (N=13). Chemical concentrations were as follows: KCl=50 mM; sucrose=100 mM; *myo*-inositol=100 mM; salicin=10 mM; and caffeine=10 mM. Dotted lines indicate the GRN responses to 50 mM KCl and bars indicate the standard errors of the mean

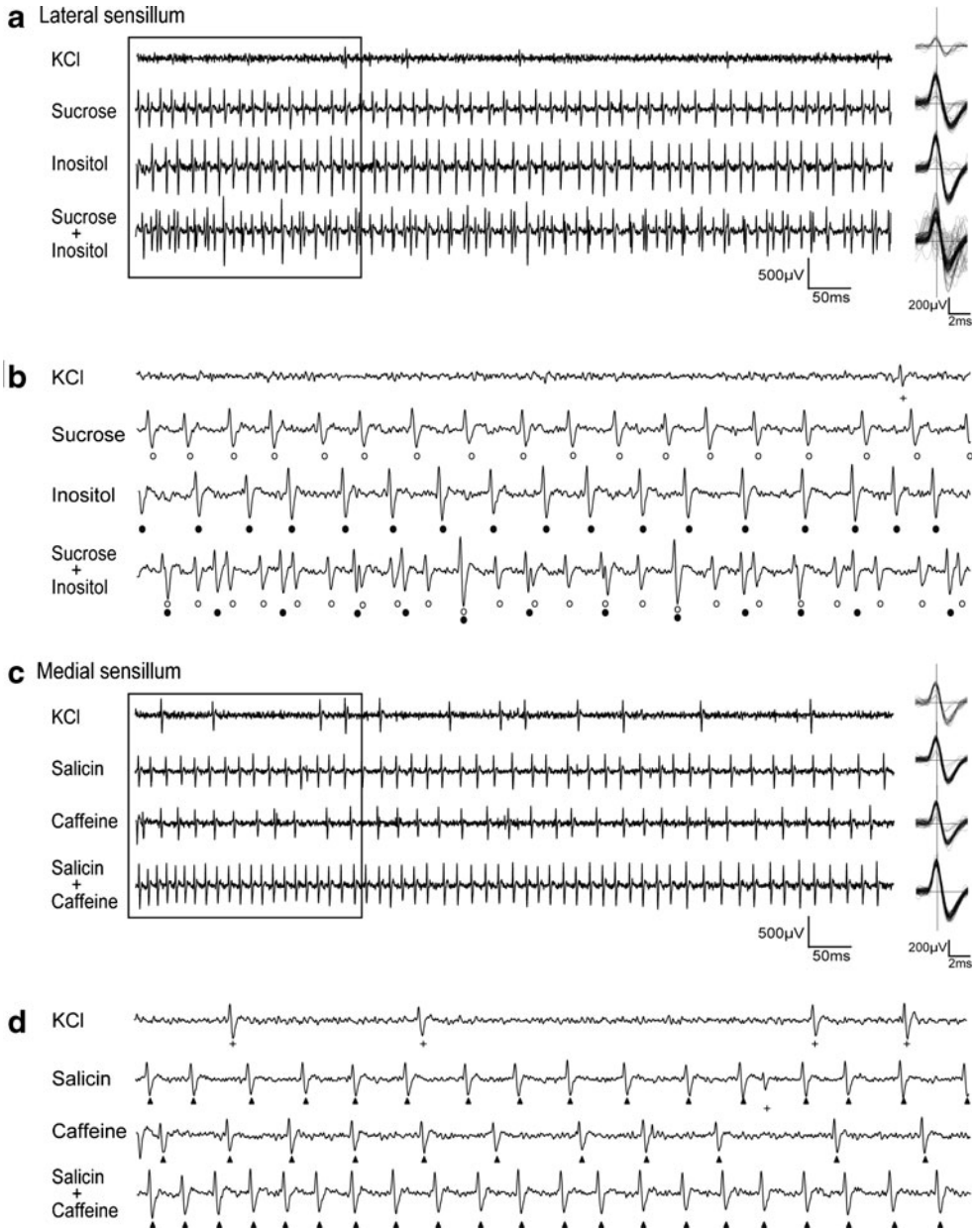

Fig. 6 Gustatory receptor neurons (GRN) responses of *Bombyx mori* styloconic sensilla to individual compounds and binary mixtures. **a** Representative traces, with spike shape superimposition, from lateral styloconic sensillum during the first second of stimulation with sugars. **b** Magnification of the above traces showing the types of spikes during the first 300 ms (box in **a**). **c** Representative traces, with spike shape superimposition, from medial styloconic sensillum during the first second of stimulation with bitter compounds. **d** Magnification of the above traces showing the types of spikes during the first 300 ms (box in **c**). Distinct spike types are indicated by empty and filled circles, crosses and filled triangles. Double labelling indicates the simultaneous occurrence of two spikes. The concentration of KCl was 50 mM and the concentrations of all other compounds were 10 mM. Mixtures consisted of 10 mM of each component

sensillum (Fig. 5d). The spike amplitude was higher in these recordings than in those where the sensilla were stimulated by the other test compounds or the KCl control. Salicin or caffeine generated similar spike shapes to controls (Fig. 5d).

Discussion

In the present study, we aimed to compare contact chemoreception and food acceptance in two species of Lepidoptera, the oligophagous silkworm *B. mori* and the polyphagous cotton bollworm *H. armigera*. Both species are deterred by bitter substances, and the morphology of their maxillary galea is similar. However, the physiology of the gustatory receptor neurons from the styloconic sensilla differs between the two species. We observed that in *B. mori*, two different neurons within the lateral sensillum respond to sucrose and *myo*-inositol, while in *H. armigera*, one neuron in the lateral styloconic sensillum responds to sucrose and one neuron in the medial styloconic sensillum responds to *myo*-inositol. In *B. mori*, a single neuron in the medial sensillum likely responds to both salicin and caffeine. However, we did not find any reliable neuronal responses to bitter compounds in either *H. armigera* lateral or medial styloconic sensilla.

Sugar Responses Sugars are strong phagostimulants in phytophagous insects (Chapman 2003; Schoonhoven and Van Loon 2002). In a previous study on *M. sexta,* a facultative specialist, it was shown that *myo*-inositol is detected by two neurons in each styloconic sensillum, and sucrose by a single

neuron in the lateral styloconic sensillum (Glendinning et al. 2007). We found the same for sucrose, but not for *myo*-inositol, which is detected by a single neuron in the lateral sensillum in *B. mori* or in the medial sensillum in *H. armigera* (Schoonhoven and Van Loon 2002). The finding that in *B. mori* sucrose and *myo*-inositol are detected by two different neurons is in agreement with earlier investigations (Ishikawa 1963, 1967) and leads us to infer the expression of at least two different sugar receptors in the lateral sensillum. Indeed, in *B. mori,* two sugar receptors, for *myo*-inositol and for fructose, recently have been characterized (Sato et al. 2011; Zhang et al. 2011). Contrary to *B. mori*, *H. armigera* detection of *myo*-inositol is mediated by a neuron in the medial sensillum. This finding supports that of Tang et al. (2000). Interestingly, Simmonds and Blaney (1991) reported a second sucrose responsive neuron in the medial sensillum, but did not mention the concentrations tested or whether they tested *myo*-inositol.

In this study, the concentrations of sucrose and *myo*-inositol that generated strong responses bracketed the levels found in mulberry leaves and used in artificial diet (Ito 1960, 1967). *Myo*-inositol has been reported to be an essential nutrient for *B. mori* (Ito 1967), and has been included in media for lepidopteran cell culture since Grace (Grace 1962). In *B. mori*, *myo*-inositol elicits prolonged feeding, but does not act as an initiator (Hamamura et al. 1962). This contrasts with the response of *Manduca sexta* to *myo*-inositol, which initiates feeding without increasing consumption (Glendinning et al. 2000). In their study on *M. sexta* host plants, Nelson and Bernays (1998) could not find any link between the levels of *myo*-inositol on the leaf surface and sugar or protein levels in the leaf tissue. They noted that the highest concentration of *myo*-inositol in leaves was approximately 1 mM. Here, however, we show neural responses to 10^{-3} mM *myo*-inositol in both species, albeit stronger in *H. armigera*, bringing them within the physiological range and supporting the hypothesis that *myo*-inositol plays a role in initiating feeding in nature.

Bitter Responses Food acceptance by phytophagous insects is determined partly by detection of potentially deterrent secondary plant compounds (Schoonhoven et al. 2005). In this study, we used the deterrents salicin and caffeine, both bitter to humans, which are from two different chemical classes (ß-glycoside and alkaloid, respectively). They have been used commonly in experiments with lepidopterans such as *B. mori* and *M. sexta* (Blaney and Simmonds 1988; Glendinning et al. 2006; Ishikawa 1966; Ishikawa and Hirao 1963). Both *B. mori* and *H. armigera* were inhibited from feeding by caffeine or salicin at naturally occurring concentrations. This is consistent with previous findings that *B. mori* rejects food containing salicin and has a "bitter substance detector" (Ishikawa 1966). *Helicoverpa armigera* is much less behaviorally sensitive to salicin, but more sensitive to caffeine than *B. mori*. The results reported here may suggest a more complex relationship between antifeeding effects and the identities and concentrations of secondary compounds likely to be encountered. The generalization is known that generalists are behaviorally less sensitive to deterrent compounds than specialists (Bernays et al. 2000). However, our findings could provide new information on the behavioral responses in these two species.

We also found that only *H. armigera*'s behavioral sensitivity to salicin occurred at higher levels than those found in natural rich sources. *Helicoverpa armigera* fifth instars indeed were able to grow and pupate normally when fed on 10 mM salicin (24/24 survived to the adult stage, data not shown), which is at the upper end of the concentrations found in nature. The result confirms that this species is either highly tolerant of salicin or has potent detoxifying mechanisms for it. As the behavioral test involves exposing larvae to the bitter compounds for 24 hr, we could not exclude the possibility that feeding inhibition is partly mediated through a post-ingestive toxicity mechanism. However, the behavior observed on initial encounter with bitter substances indicated that feeding inhibition is substantially driven by sensory properties of the antifeedants.

Additionally, the previous observations reporting the presence in *B. mori* of a GRN in the medial styloconic sensillum that responds to salicin, indicates that it may contribute to the behavioral response (Asaoka 2000; Ishikawa 1963, 1966; Ishikawa and Hirao 1963). However, 1–10 mM salicin has also been shown to activate a deterrent cell in the epipharyngeal sensilla (Asaoka and Shibuya 1995), so it is possible that this cell in combination with others in the styloconic sensillum (Dethier 1937, 1973; Descoins and Marion-Poll 1999; Mori 1982; Tanaka et al. 1994) contributes to the behavioral effect. There was a profound difference between the sensory and behavioral responses of *B. mori* to salicin and caffeine, which could involve as yet non-obvious differences in firing patterns, or implicate the involvement of other sensory mechanisms. In *H. armigera* styloconic sensilla, we saw no reliable neuronal responses to caffeine and salicin, suggesting that other sensory organs such as the maxillary palps or epipharyngeal sensilla may be involved in the detection of these compounds, as was reported in *M. sexta* where three neurons located in the epipharyngeal, lateral and medial styloconic sensilla responded to both salicin and caffeine (Glendinning et al. 2006). Interestingly, the initial response of *H. armigera* to deterrent compounds involves more biting than in *B. mori* where drumming seemed to predominate (see Online Resource 2, 3).

Relationship Between Gustatory Sensitivity and Host Range Based on the current study and the spatial pattern of GRNs in styloconic sensilla responding to various taste stimuli in lepidopterans with different feeding habits (Schoonhoven

and Van Loon 2002), it is clear that larvae are likely to express several gustatory receptors specific for various sugars and bitter compounds. It also suggested that, at least in this case, species with different feeding preferences express functionally equivalent gustatory receptors in different locations in the medial and lateral styloconic sensilla. These differences may reflect selective pressures to detect or discriminate a different spectrum of plant compounds for each species. Besides, more complex determinants that may include host range, metabolic capacity and gustatory repertoire could multiply mediate larval sensing of specific plant secondary compounds.

Acknowledgments We thank Chengyuan Yang, Lena Sun, and Lin Tang (State Key Laboratory of Silkworm Genome Biology, Southwest University, China) for technical assistance. We thank Owain Edwards (CSIRO Ecosystem Sciences, Australia) and Frédéric Marion-Poll (INRA, France) for comments on a draft of this manuscript. We also thank two anonymous reviewers for suggestions that improved this paper. This work was supported by National High-tech R&D Program of China (863 Program), National Program on Key Basic Research Project (973 Program) and grants from CSIRO Australia Appropriation Funds.

References

Asaoka K (2000) Deficiency of gustatory sensitivity to some deterrent compounds in "polyphagous" mutant strains of the silkworm, *Bombyx mori*. J Comp Physiol A 186:1011–1018

Asaoka K, Shibuya T (1995) Morphological and electrophysiological characteristics of the epipharyngeal sensilla of the silkworm, *Bombyx mori*. Entomol Exp Appl 77:167–176

Bernays EA, Chapman RF (1987) The evolution of deterrent responses in plant-feeding insects. In: Chapman RF, Bernays EA (eds) Perspectives in chemoreception and behavior. Springer, New York, pp 159–173

Bernays EA, Chapman RF (1994) Host-plant selection by phytophagous insects. Chapman & Hall, New York

Bernays EA, Chapman RF (2000) A neurophysiological study of sensitivity to a feeding deterrent in two sister species of *Heliothis* with different diet breadths. J Insect Physiol 46:905–912

Bernays EA, Oppenheim S, Chapman RF, Kwon H, Gould F (2000) Taste sensitivity of insect herbivores to deterrents is greater in specialists than in generalists: a behavioral test of the hypothesis with two closely related caterpillars. J Chem Ecol 26:547–563

Blaney WM, Simmonds MSJ (1988) Food selection in adults and larvae of three species of Lepidoptera: a behavioural and electrophysiological study. Entomol Exp Appl 49:111–121

Chapman RF (1982) Regulation of food intake by phytophagous insects. In: Addink ADF, Spronk N (eds) Exogenous and endogenous influences on metabolic and neural control. Pergamon Press, Oxford, pp 19–30

Chapman RF (2003) Contact chemoreception in feeding by phytophagous insects. Ann Rev Entomol 48:455–84

Chin C, Li L, Wei D, Wang Z (1962) Some characteristics in the food preference and nutrition of the cotton bollworm. Acta Entomol Sin 11:327–340 (In Chinese)

Descoins C, Marion-Poll F (1999) Electrophysiological responses of gustatory sensilla of *Mamestra brassicae* (Lepidoptera, Noctuidae) larvae to three ecdysteroids: ecdysone, 20-hydroxyecdysone and ponasterone A. J Insect Physiol 45:871–876

Dethier VG (1937) Gustation and olfaction in lepidopterous larvae. Biol Bull 72:7–23

Dethier VG (1973) Electrophysiological studies of gustation in lepidopterous larvae. J Comp Physiol 82:103–134

Devitt BD, Smith JJB (1985) Action of mouthparts during feeding in the dark-sided cutworm, *Euxoa messoria* (Lepidoptera, Noctuidae). Can Entomol 117:343–349

Eichenseer H, Mullin AM (1997) Antifeedant comparisons of GABA/glycinergic antagonists for diabroticite leaf beetles (Coleoptera: Chrysomelidae). J Chem Ecol 23:71–82

Glendinning JI, Nelson NM, Bernays EA (2000) How do inositol and glucose modulate feeding in *Manduca sexta* caterpillars? J Exp Biol 203:1299–1315

Glendinning JI, Davis A, Rai M (2006) Temporal coding mediates discrimination of "bitter" taste stimuli by the insect. J Neurosci 26:8900–8908

Glendinning JI, Jerud A, Reinherz A (2007) The hungry caterpillar: an analysis of how carbohydrates stimulate feeding in *Manduca sexta*. J Exp Biol 210:3054–3067

Grace TD (1962) Establishment of four strains of cells from insect tissues grown *in vitro*. Nature 195:788–789

Grimes LR, Neunzig HH (1986) Morphological survey of the maxillae in last stage larvae of the suborder Ditrysia (Lepidoptera): mesal lobes (Laciniogaleae). Ann Entomol Soc Am 79:510–526

Hamamura Y, Hayashiya K, Naito K, Matsuura K, Nishida J (1962) Food selection by silkworm larvae. Nature 194:754–755

Hiroi M, Meunier N, Marion-Poll F, Tanimura T (2004) Two antagonistic gustatory receptor neurons responding to sweet-salty and bitter taste in *Drosophila*. J Neurobiol 61:333–342

Hsiao T, Fraenkel G (1968) The influence of nutrient chemicals on the feeding behavior of the Colorado potato beetle, *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae). Ann Entomol Soc Am 61:44–54

Ishikawa S (1963) Responses of maxillary receptors in the larva of the silkworm, *Bombyx mori*, to stimulation by carbohydrates. J Cell Comp Physiol 61:99–107

Ishikawa S (1966) Electrical response and function of a bitter substance receptor associated with the maxillary sensilla of the larva of the silkworm, *Bombyx mori L*. J Cell Physiol 67:1–12

Ishikawa S (1967) Maxillary chemoreceptors in the silkworm. In: Hayashi T (ed) Olfaction and taste II. Pergamon Press, Oxford, pp 761–777

Ishikawa S, Hirao T (1961) Tactile sense organ on the head of silkworm larva (*Bombyx mori*). J Sericulture Sci Japan 30:17–22

Ishikawa S, Hirao T (1963) Electrophysiological studies of taste sensation in the larvae of the silkworm, *Bombyx mori*. Responsiveness of sensilla styloconica on the maxilla. Bull Seric Exp Sta Japan 19:297–357

Ishikawa S, Hirao T, Arai N (1969) Chemosensory basis of hostplant selection in the silkworm. Entomol Exp Appl 12:544–554

Ito T (1960) Effect of sugars on feeding of larvae of the silkworm, *Bombyx mori*. J Insect Physiol 5:95–107

Ito T (1967) Nutritional requirements of the silkworm, *Bombyx mori L*. Proc Jpn Acad 43:57–61

Jermy T (1964) Feeding inhibitors and food preference in chewing phytophagous insects. Entomol Exp Appl 9:1–12

Legay JM (1958) Recent advances in silkworm nutrition. Ann Rev Entomol 3:75–86

Lindroth RL, Scriber JM, Hsia MTS (1988) Effects of the quaking aspen compounds catechol, salicin and isoniazid on 2 subspecies of tiger swallowtails. Am Midl Nat 119:1–6

Ma WC (1972) Dynamics of feeding responses in *Pieris brassicae Linn* as a function of chemosensory input: A behavioural, ultrastructural and electrophysiological study. Mededelingen landbouwhogeschool Wageningen, Nederland, pp 72–11

Mahon RJ, Olsen K, Downes SJ, Addison S (2007) Frequency of alleles conferring resistance to the Bt toxins Cry1Ac and Cry2Ab in Australian populations of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). J Econ Entomol 100:1844–1853

Marion-Poll F, van der Pers JNC (1996) Un-filtered recordings from insect taste sensilla. Entomol Exp Appl 80:113–115

Mori M (1982) n-Hexacosanol and n-octacosanol: feeding stimulants for larva of the silkworm, *Bombyx mori*. J Insect Physiol 28:969–973

Nathanson JA (1984) Caffeine and related methylxanthines - possible naturally-occurring pesticides. Science 226:184–187

Nelson N, Bernays EA (1998) Inositol in two host plants of *Manduca sexta*. Entomol Exp Appl 88:189–193

Sato K, Tanaka K, Touhara K (2011) Sugar-regulated cation channel formed by an insect gustatory receptor. Proc Natl Acad Sci USA 108:11680–11685

Schoonhoven LM (1969) Gustation and food plant selection in some lepidopterous larvae. Entomol Exp Appl 12:555–564

Schoonhoven LM (1987) What makes a caterpillar eat? The sensory codes underlying feeding behaviour. In: Chapman RF, Bernays EA (eds) Advances in chemoreception and behavior. Springer, New York, pp 69–77

Schoonhoven LM, Blom F (1988) Chemoreception and feeding behaviour in a caterpillar: towards a model of brain functioning in insects. Entomol Exp Appl 49:123–129.

Schoonhoven LM, van Loon JJA (2002) An inventory of taste in caterpillars: each species its own key. Acta Zool Acad Sci Hung 48(suppl1):215–263

Schoonhoven LM, Van Loon JJA, Dicke M (2005) Host-plant selection: How to find a host plant. In: Insect-plant biology. Oxford University Press, New York, pp 136–160

Shields VDC (2009) Fine structure of the galeal styloconic sensilla of larval *Lymantria dispar* (Lepidoptera: Lymantriidae). Ann Entomol Soc Am 102:1116–1125

Simmonds MSJ, Blaney WM (1991) Gustatory codes in lepidopterous larvae. Symp Biol Hung 39:17–27

Smiley JT, Horn JM, Rank NE (1985) Ecological effects of salicin at 3 trophic levels—new problems from old adaptations. Science 229:649–651

Tanaka Y (1943) Santai-serrigaku. Meiliun-do, Tokyo (In Japanese)

Tanaka Y, Asaoka K, Takeda S (1994) Different feeding and gustatory responses to ecdysone and 20-hydroxyecdysone by larvae of the silkworm, *Bombyx mori*. J Chem Ecol 20:125–133

Tang D, Wang C, Luo L, Qin J (2000) Comparative study on the responses of maxillary sensilla styloconica of cotton bollworm *Helicoverpa armigera* and oriental tobacco budworm *H. assulta* larvae to phytochemicals. Sci China Ser C 43:607–612

Teakle RE, Jensen JM (1985) *Heliothis punctigera*. In: Singh R, Moore RF (eds) Handbook of insect rearing, vol. 2. Elsevier, Amsterdam, The Netherlands, pp 312–322

Thorsterinson AJ (1960) Host selection in phytophagous insects. Ann Rev Entomol 5:193–218

van Loon JJA (1990) Chemoreception of phenolic acids and flavonoids in larvae of two species of *Pieris*. J Comp Physiol 166:889–899

Waldbauer GP, Fraenkel G (1961) Feeding on normally rejected plants by maxillectomized larvae of the tobacco hornworm, *Protoparce sexta* (Lepidoptera, Sphingidae). Ann Entomol Soc Am 54:477–485

Xia Q, Zhou Z, Lu C, Cheng D, Dai F, Li B et al (2004) A draft sequence for the genome of the domesticated silkworm (*Bombyx mori*). Science 306:1937–40

Xu M, Zhang G, Zhu H (1958) A study on the cotton bollworm. Chin J Appl Entomol 1:18–30 (In Chinese)

Zhang H, Anderson A, Trowell S, Luo A, Xiang Z, Xia Q (2011) Topological and functional characterization of an insect gustatory receptor. PLoS One 6:e24111